



## Cellulose and Chitosan Composite Membranes for Protein and Salt Filtration

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### ABSTRACT

Cellulose membrane (SE) was prepared by culturing *Acetobacter xylinum* in a media with sucrose as a carbon source and was used as a supporting membrane in this study. Pore size of the supporting membrane was studied by means of molecular weight cut off (MWCO) and SEM micrographs. For making cellulose/chitosan composite membrane SE/CH, chitosan solution was used as a coating polymer and applied by a casting method. This resulted in a smaller hydraulic permeability coefficient ( $L_p$ ) from  $6.7 \times 10^{-11} \text{ m}^3 \text{ N}^{-1} \text{ s}^{-1}$  in membrane SE to  $1.94 \times 10^{-12} \text{ m}^3 \text{ N}^{-1} \text{ s}^{-1}$  in the composite membrane SE/CH. Using PEG of several molecular weights as feed solution, the MWCO of the SE membrane was 200 kDa while that of the SE/CH membrane was 6 kDa. The former rejected  $1 \text{ g L}^{-1}$  BSA by 80%. With pH between 3 and 8, the composite membrane SE/CH rejected NaCl and  $\text{NaHCO}_3$  by 50%, independent of the pH level. However, when using a divalent salt solution of  $\text{MgSO}_4$  the rejection was increased up to 85%, with an optimum at pH 6–7 and a permeate flux of  $5.0 \text{ L m}^{-2} \text{ h}^{-1}$  at pressures of 0.5 MPa.

**Keywords:** Cellulose, chitosan, membrane, water flux, BSA, salt, filtration

### 1.0 INTRODUCTION

Cellulose is one of the most abundant biological polymers in nature. One of its well known derivatives that has been used since the beginning of filtration technology is cellulose acetate. Generally, pure cellulose can be produced in laboratory by bacteria; such as *Acetobacter xylinum*, *Rhizobium*, *Alcaligenes*, *Agrobacterium*, *Sacina*, *Pseudomonas*, has been used in several applications [1, 2]. However, *A. xylinum* is the bacterium most used, due to its high cellulose production compared to others. In addition, bacterial cellulose possesses good water-binding capacity, high elasticity and high tensile strength [1]. Chitosan is another biological polymer derived from chitin, which is a polysaccharide found in the exoskeletons of crustaceans and is found in a wide range of natural sources like crab and shrimp shells. Due to high affinity to water and good mechanical and chemical

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stability, chitosan is widely used as a selective layer for composite membranes in filtration technology [3, 4].

In many cases, a composite membrane was made by casting a hydrophilic polymer onto the surface of a base membrane, followed by cross-linking of the thin coating layer with glutaraldehyde [4, 5] for strengthening purposes. This paper firstly investigates the properties of cellulose membrane produced by *A. xylinum* using sucrose and glucose as a carbon source for a comparison. Secondly, chitosan/cellulose composite membrane was prepared. Membrane characterization parameters such as hydraulic permeability, MWCO, and tests on bovine serum albumin (BSA) and salt rejection are reported.

## 2.0 MATERIALS AND METHODS

### 2.1 Preparation of Membranes

Two types of biomaterials were used for membrane preparation. They were cellulose produced from bacteria *A. xylinum* (TISTR 975, Thailand) and chitosan (Sea Fresh, 400 kDa deacetylation 76%). The former was prepared by culturing the cells to  $1 \times 10^6$  cfu ml<sup>-1</sup> density in a media composed of 4.0% (w/v) sucrose, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.033% (w/v) Na<sub>2</sub>HPO<sub>4</sub> and 0.0115% (w/v) citric acid, pH 4. The culture was incubated statically for 3 days at 27°C in a laminar flow incubator. The produced cellulose accumulated at the surface of the culture medium. After a design period of time, the obtained cellulose membrane was referred to as SE and was also used as a supporting membrane in this study. Chitosan solution was prepared following Musale and Kumar [5] by dissolving chitosan flakes in a mixture between 100 ml of 0.3 M acetic acid and 100 ml of 0.2 M sodium acetate, to make up a 1% chitosan solution. About 0.5 ml of the solution was poured over a supporting cellulose SE membrane disk (4.7 cm diameter) and a thin chitosan membrane was formed by slip casting method at room temperature. After drying in a dust free area, the membrane was immersed in a 0.01% (w/v) glutaraldehyde in phosphate buffer for 1 hr, then washed and dried at room temperature again to cross-link the composite membrane. This was referred to as membrane SE/CH.

### 2.2 Membrane Characterization

Hydraulic permeability ( $L_p$ ) was estimated from a slope between water flux and the applied pressure of a membrane mounted in the dead end unit (Figure 1). Distilled water was used as a feed and permeate volume was collected after known periods of time. Filtration of bovine serum albumin (BSA) solution was made using membrane SE under a 100 kPa applied pressure. The concentration of BSA on the feed side was 1 g L<sup>-1</sup> at pH 6.35. From the known protein concentration of the feed

( $C_f$ ) and the permeate ( $C_p$ ), rejection from the membrane could be estimated using  $\left(1 - \frac{C_p}{C_f}\right) \times 100$ .

Each concentration of BSA was deduced from a standard graph using a spectrophotometer at 750 nm wavelength. Similarly, salt rejection from membrane SE/CH was studied at pH 3-8 under 0.8 MPa, using the same concentration as BSA. The amount of salt was determined in terms of solution conductivity (Tetracon 325, LF 318, USA). Filtration of NaCl, NaHCO<sub>3</sub> and MgSO<sub>4</sub> solution was made for a comparison at 800 kPa, the same pressure as used by Runhong and Zho (2004). In all cases, the feed was continuously magnetic stirred to avoid precipitation on the membrane surface.

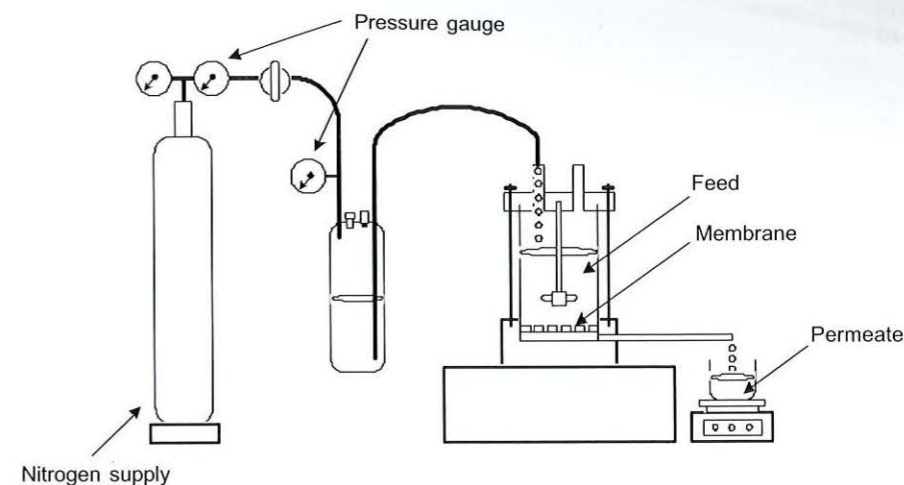


Figure 1 Schematic representation of a dead end filtration unit

To determine pore size of the dense composite membrane SE/CH, 50 ppm of PEG (polyethylene glycols) aqueous solution of various MW (0.9 – 10 kDa) were used as a feed. Starting with a feed volume of 100 ml and 0.8 MPa, concentration of a permeate volume was determined using a spectrophotometer at 535 nm wavelength. Pore size of the membrane was determined as a MWCO value when PEG rejection reached at least 90% [6].

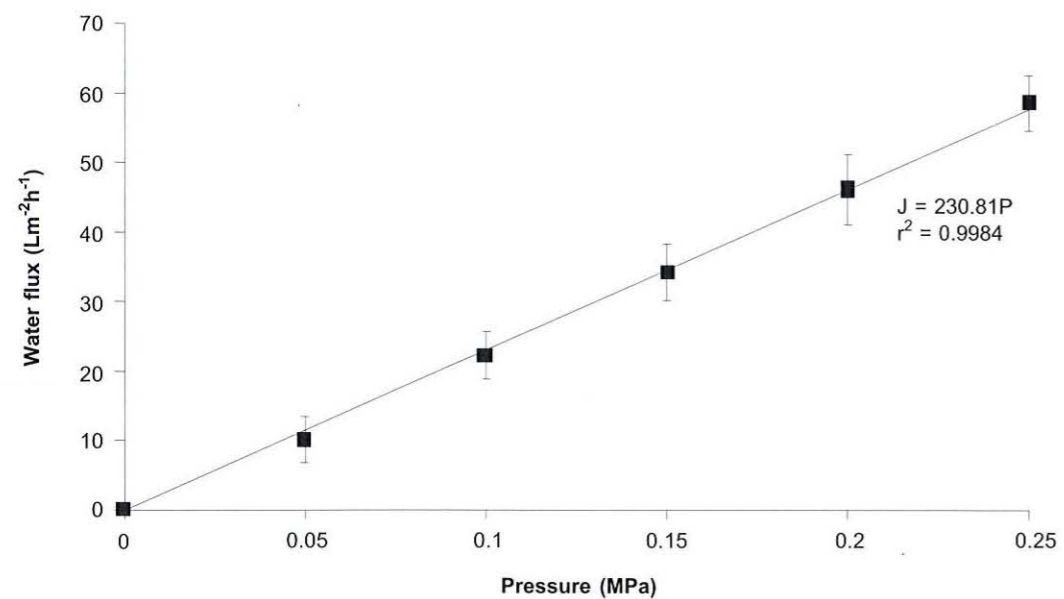
## 3.0 RESULTS AND DISCUSSION

### 3.1 Hydraulic Permeability Coefficient ( $L_p$ )

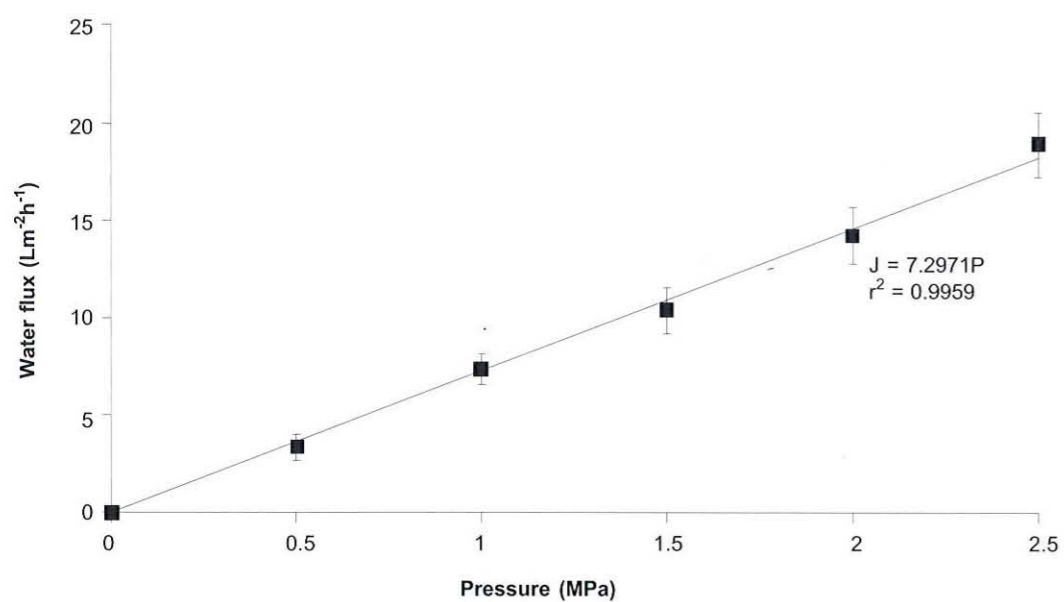
This study showed that membrane SE could stand a compressive pressure in the dead end unit up to 0.25 MPa without breaking and provided permeate water at a rate of 60 L m<sup>-2</sup> h<sup>-1</sup>. Figure 2 shows that the fluxes are linearly dependent on the pressure and water fluxes of membrane SE/CH is smaller than that of SE at every pressure used. The greater flux lead to the larger  $L_p$  value of membrane SE and it was  $67 \times 10^{-12}$  m<sup>3</sup> N<sup>-1</sup> s<sup>-1</sup>, while that of membrane SE/CH was  $2 \times 10^{-12}$  m<sup>3</sup> N<sup>-1</sup> s<sup>-1</sup>. Based on the  $L_p$  value between  $2.6 \times 10^{-11}$  –  $4.0 \times 10^{-10}$  m<sup>3</sup> N<sup>-1</sup> s<sup>-1</sup> [7], the membranes SE is classified as a UF membrane. The  $L_p$  value falls between  $9.2 \times 10^{-13}$  –  $5.0 \times 10^{-12}$  m<sup>3</sup> N<sup>-1</sup> s<sup>-1</sup> for membrane SE/CH is of NF-type [8-10].

### 3.2 Membrane Molecular Weight Cut Off (MWCO)

The PEG content in the permeate volume was analyzed when PEG solutions of several different molecular weights were used as feed. Figure 3(a) shows that PEG rejection from the membrane SE increases with the molecule size and the rejection reached 90% at about 200 kDa. The PEG permeate in Figure 3(b) is constant during 5–30 min of filtration period with permeate flux value scattering around 15 L m<sup>-2</sup> h<sup>-1</sup>, regardless of the molecular weight used. Figure 4 also shows the increase in PEG rejection of the membrane SE/CH with the size of PEG used and the 90% rejection point is found at about 6 kDa. SEM micrographs of these membranes are compared in Figure 5. These confirm

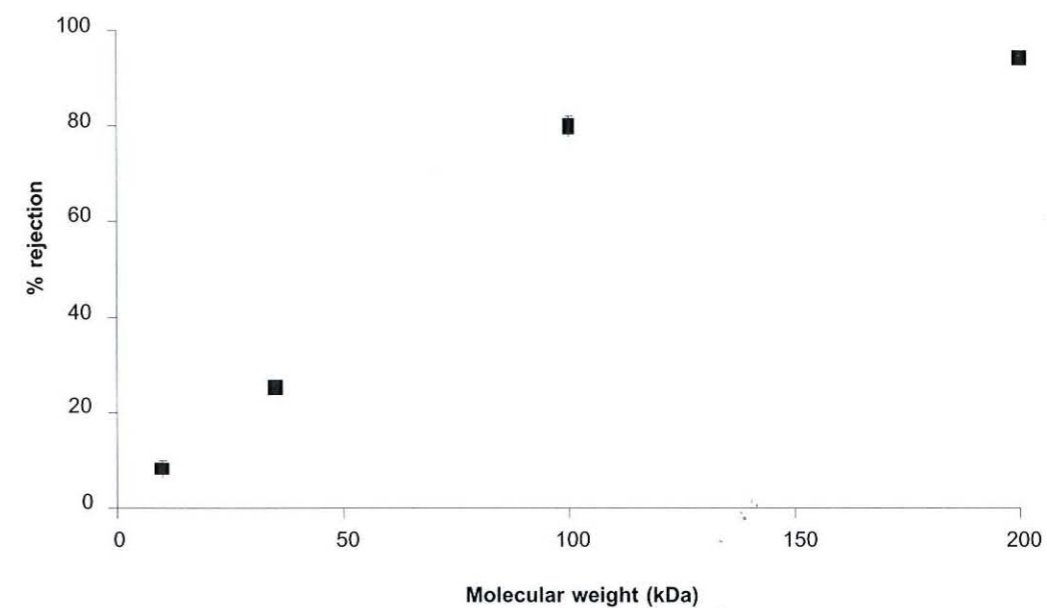


(a)

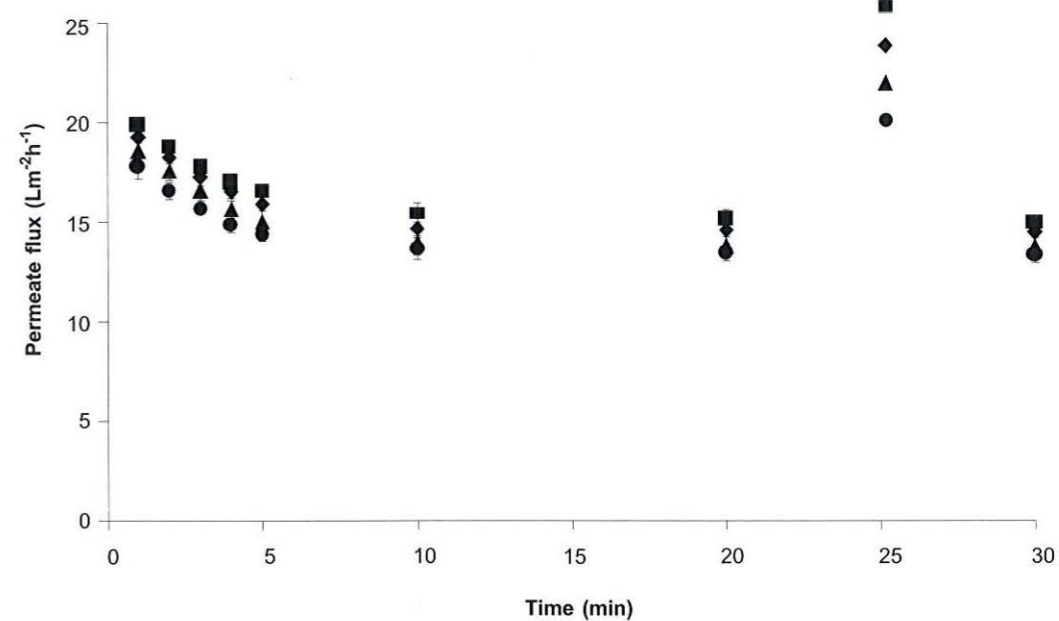


(b)

**Figure 2** Water fluxes against applied pressures of membrane SE (a) SE/CH (b) Each data was averaged from 3 experiments



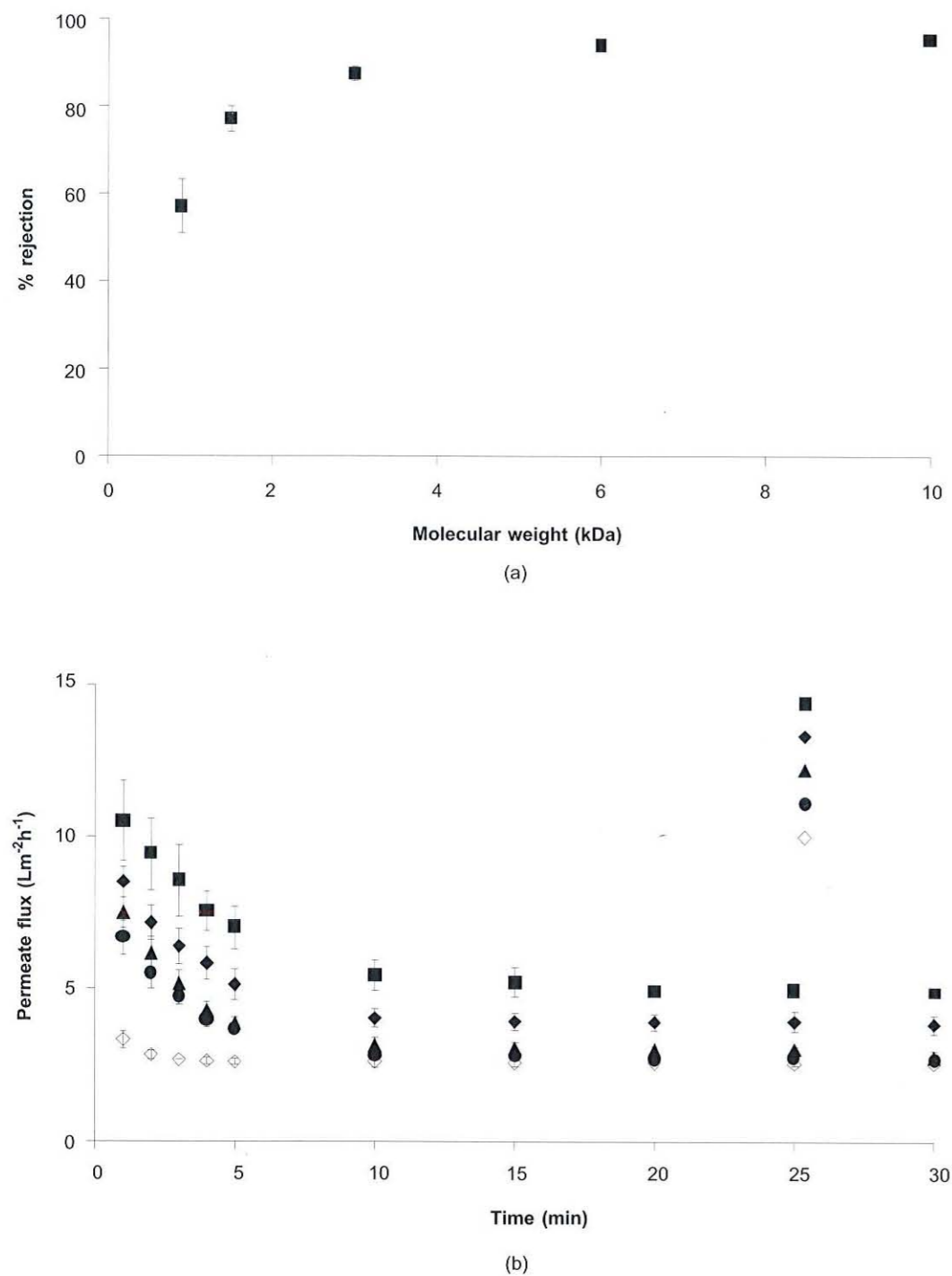
(a)



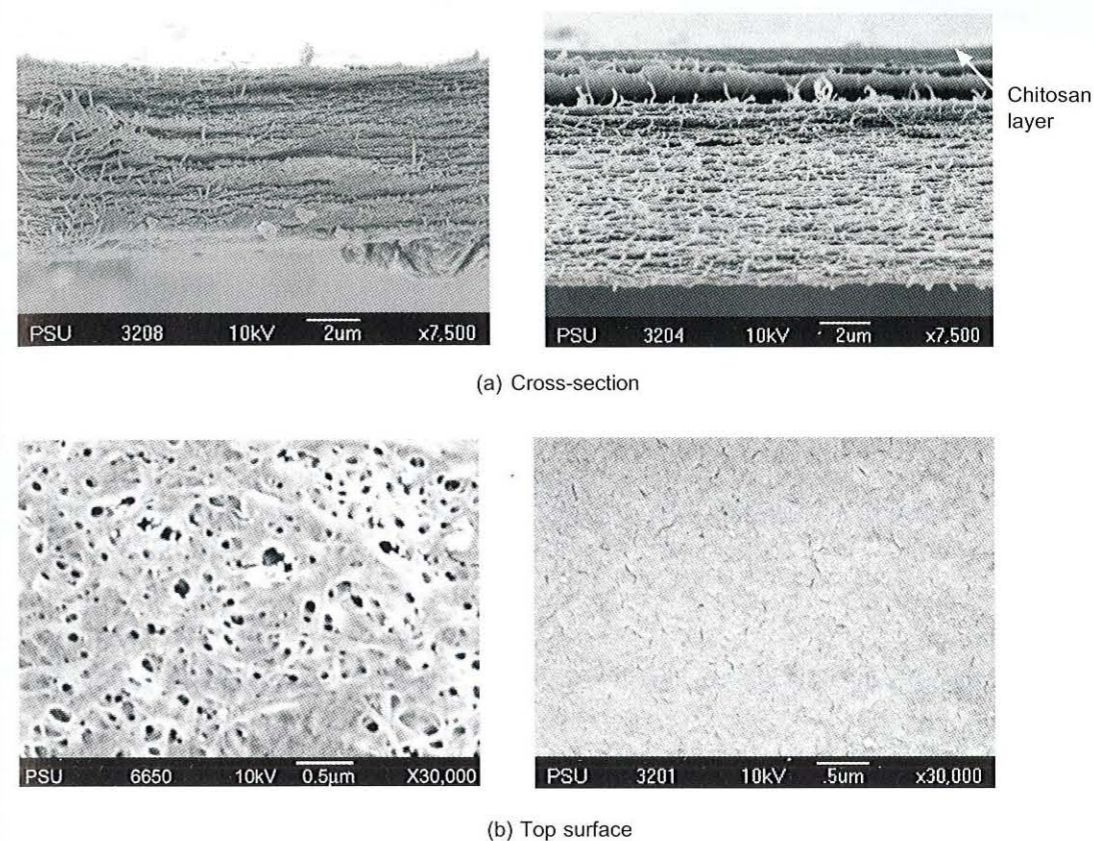
(b)

**Figure 3** Filtration of several PEG molecular weights for membrane SE at 100 kPa. (a) Rejection of PEG and (b) Permeate flux. Each data was averaged from 3 experiments





**Figure 4** Filtration of several PEG molecular weights for membrane SE/CH at 800 kPa. (a) Rejection of PEG and (b) Permeate flux. Each data was averaged from 3 experiments



**Figure 5** SEM micrographs of (a) cross-section (b) top surface of two membrane types

the smaller pore size of the composite membrane SE/CH. As proposed by Puhlfürb *et al.* [11], the equation  $R = 0.167(MW)^{0.557}(\text{Å})$  and  $R = 0.262(MW)^{1/2} - 0.3(\text{Å})$  were utilized to estimate pore size of 2–40 kDa membranes and 200–3000 kDa membranes, respectively. The 6 kDa pore size of membrane SE/CH was equivalent to 2.1 nm and the 200 kDa of membrane SE was 116 Å or 0.01 μm. These calculated values agree well with a schematic representation of pore size for membrane separation process described by Baker [12].

### 3.3 Filtration of BSA by Membrane SE

The permeate flux of BSA is shown in Figure 6 using 1.5 MPa pressure on the feed side. The flux drops immediately after the start of filtration, indicating pore blockage of the membrane. However, it remains stable after about 5 min of the filtration period. Although the globular size of BSA is 64 Å [12], much smaller than the above calculated pore size (116 Å), the protein would be either thread-like or being clump after dissolved in a solution, depending on solution pH. During the 5 min filtration, some BSA would obstruct the passage, leading to a reduction of BSA penetration at a later stage. It was found that BSA rejection of membrane SE was  $83.1 \pm 0.7\%$ . Permeate flux of the membrane is  $15 \text{ L m}^{-2} \text{ h}^{-1}$  much less than that of water (see Figure 2(a)), due to a smaller pressure was used.



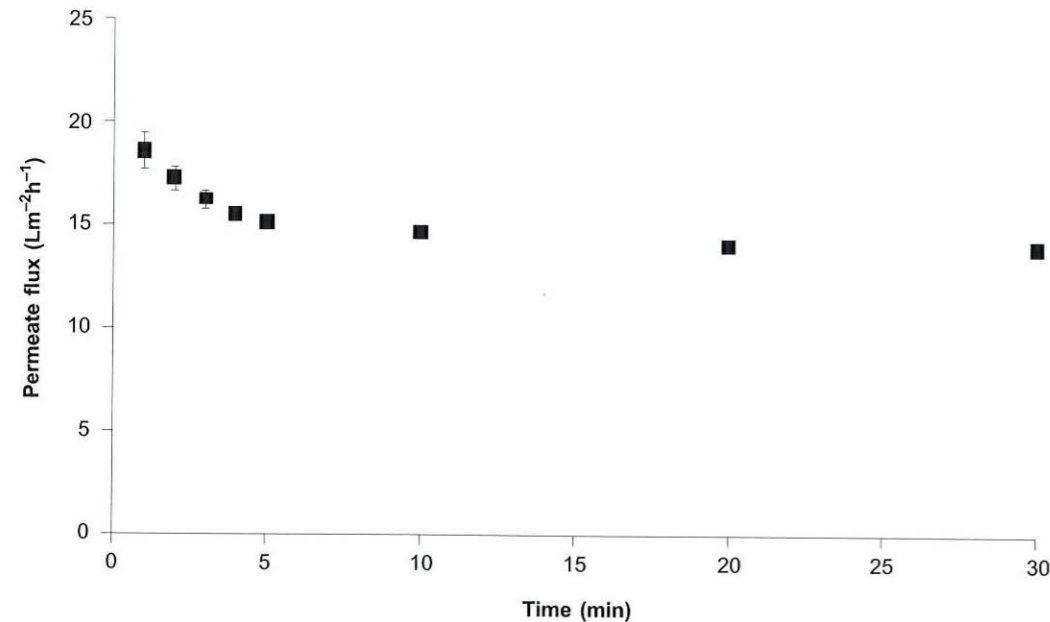
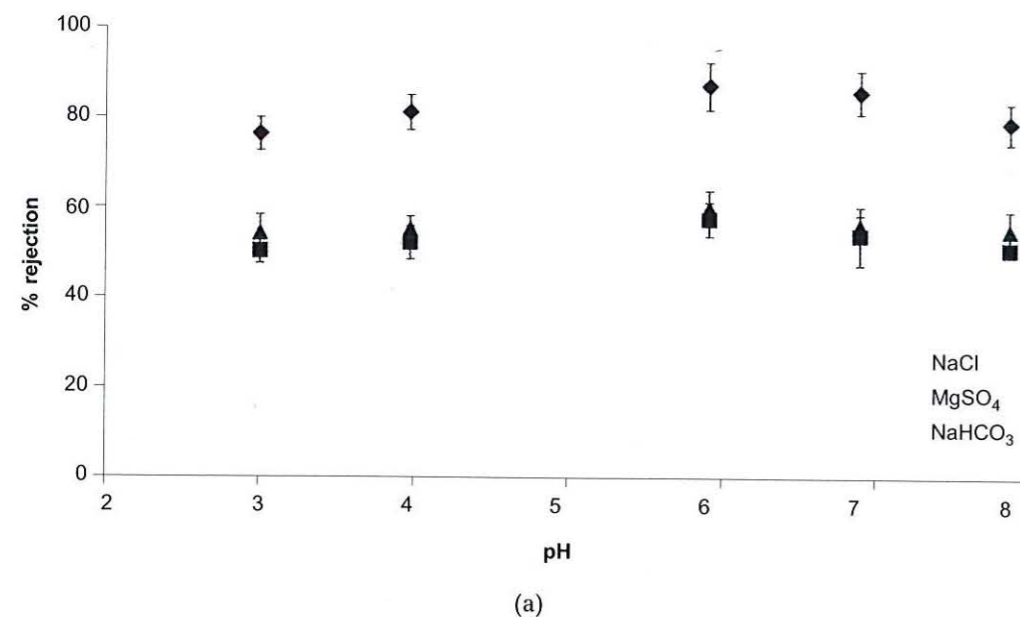


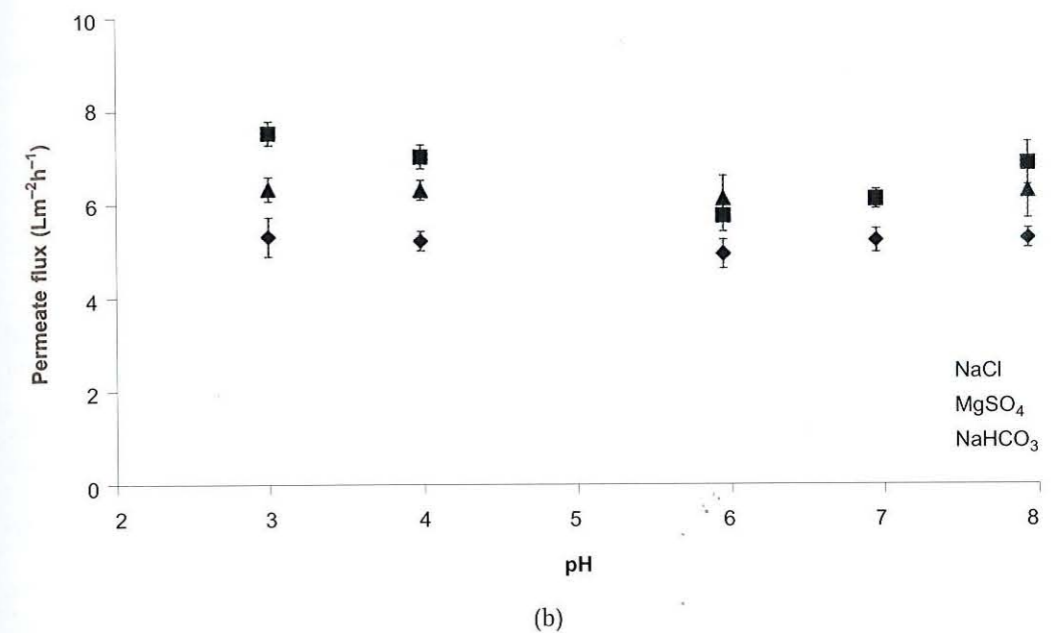
Figure 6 Permeate flux for BSA filtration using membrane SE. Data was averaged from 3 experiments

### 3.4 Filtration of Salts by Composite Membrane SE/CH

Figure 7(a) shows the effect of pH on salt rejection of dense composite membrane SE/CH. The rejections of NaCl and NaHCO<sub>3</sub> from the membrane are nearly the same at all pH levels, with the



(a)



(b)

Figure 7 Effect of pH level on rejection (a) and permeate flux (b) of NaCl, NaHCO<sub>3</sub> and MgSO<sub>4</sub> by SE/CH composite membrane. Each data was averaged from 3 experiments

value of about 50%. However, with a divalent salt MgSO<sub>4</sub> the rejection is increased to 80% at pH 3 and slightly increases further to about 85% at pH between 6 and 7. Figure 7(b) shows that the permeate fluxes of monovalent salts are greater at all pH levels. Averaging over the pH range, it was  $6.36 \pm 0.53 \text{ L m}^{-2} \text{ h}^{-1}$  for the monovalent salts and  $5.0 \pm 10.18 \text{ L m}^{-2} \text{ h}^{-1}$  for the divalent salt. This result together with the calculated MWCO value implies that the composite membrane is a NF membrane type. It should be noted that cellulose and chitosan are hydrophilic materials. As a composite membrane, it absorbed water to its maximum weight of about 120% after 8 minutes. Under testing method, it would absorb salt solution readily and the amount of salt found in the permeate side was from both pressure driven and diffusion process. The higher rejection of MgSO<sub>4</sub> salt could be due to its larger molecular weight compared to NaCl. The result indicated that the separation property to the composite membrane SE/CH to these salts was related to the pore size of the chitosan film.

### 4.0 CONCLUSION

Bacterial cellulose produced by *A. xylinum* and cellulose/chitosan composite membranes show potential in filtration technology. The pure cellulose membrane is an ultrafiltration type which rejects protein by 83% at 500 kPa. After being coated with a dilute chitosan solution, the pore size of the cellulose/chitosan was reduced from 200 kDa to 6 kDa. The composite membrane rejects divalent salt and monovalent salts by 85% and 50%, respectively, with optimum performance at pH 6-7.

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